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Meniscal transection rather than excision increases pain behavior and structural damage in experimental osteoarthritis in mice

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SUMMARY

Objective: To evaluate pain behavior and structural damage in mice subjected to either meniscal transection or removal.
Methods: Mice (10/group) were subjected to transection of the medial collateral and anterior cruciate ligaments (ACLT/MCLT) followed by either transection (meniscotomy) or removal (meniscectomy) of the medial meniscus. A control group was subjected only to transection of the ligaments. Pain was assessed using the electronic pressure-meter paw test. Cell influx, measured in joint exudates, and joint histopathology were assessed after 49 days. Four other groups subjected to meniscotomy received indomethacin, the inducible nitric oxide synthase (iNOS) inhibitor 1400W, morphine or the vehicles.
Results: Both meniscotomy and meniscectomy groups displayed persistent and significant increase in pain behavior as compared to controls, being significantly more severe in the former. Cell influx was more intense in the meniscotomy as compared to the meniscectomy group. Structural damage at the tibia, but not at the femur, was also more severe in the meniscotomy group. Indomethacin and 1400W partially but significantly reduced pain whereas morphine abrogated pain behavior in meniscotomized mice.
Conclusion: Meniscal transection rather than resection promotes more severe pain and structural damage in mice. Administration of opioids, cyclooxygenase and nitric oxide (NO) synthase inhibitors provide analgesia in this model. Careful description of the structures damaged is crucial when reporting experimental osteoarthritis (OA).

Introduction

The menisci are semilunar discs composed of fibrocartilaginous tissue located inside the knee joint. Their major function is to minimize damage to the articulating surfaces by absorbing shock during movement[1,2].

Meniscal damage is one of the risk factors associated with knee osteoarthritis (OA) development. Partial or complete meniscectomy ranks high among the causes for orthopedic knee surgery and is associated with OA development. However, controversy as to whether meniscal removal, meniscal repair or conservative management is the best approach to treat a clinically symptomatic torn meniscus still remains[3,4]. Presence of nerve endings in peripheral portions of the menisci may contribute to joint pain. In addition, damage to the underlying cartilage and subchondral bone could provoke bone marrow edema thus leading to pain. In these situations, preserving as much meniscus as possible could allegedly protect the joint from major damage[3,5].

Surgically-induced models are considered the best current approach to study pain development in experimental OA[6]. Previously, rats subjected to partial medial meniscectomy were shown to

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display mechanical hyperalgesia and tactile allodynia[7]. Gait changes and pain behavior were shown to be less severe in rats subjected to anterior cruciate ligament transection and partial medial meniscectomy as compared to rats subjected to the monoiodoacetate injection OA model[8]. A detailed previous study has evaluated the time course of radiographic and histological damage to the knee joint in mice exposed to four types of injury, showing that OA progression depends on the severity of the instability provoked[9]. The pain component and histological damage in the destabilizing medial meniscus (DMM) procedure in mice have been described[10,11]. In the DMM model, endogenous opioids were shown to be involved in the pain behavior[11] and the administration of a soluble nerve growth factor receptor provided analgesia[12]. However, the issue of whether surgically removing as opposed to transecting the meniscus, without removal, affects both pain behavior and histological severity has not been appreciated. Surgical procedures, especially in small animals, are aggressive thus rendering a potential risk of secondary lesions after opening the joint for complete removal of the meniscus. Therefore, special care is needed when trying to compare strategies to sever the meniscus in mice[13–15].

In the present study, we examined the differences regarding functional (pain behavior) and structural damage (histopathology) to the knee joints between mice subjected to either complete removal of the medial meniscus (meniscectomy) or complete transection without removal of the meniscus (meniscotomy) as well as the contribution of prostaglandins, nitric oxide (NO), and opioids to the pain behavior.

Materials and methods

Animals

Swiss male mice (25–30 g) were provided by the central animal house of the Federal University of Ceará, Fortaleza – CE, Brazil. Animals were housed in cages (6/cage) in temperature-controlled rooms with a 12 h light/dark cycle with free access to water and food. At the start of any experiments, mice were 2.5 months of age. All efforts were made to minimize animal suffering and the number of animals used. The protocol was approved by our local ethics committee (Comitê de Ética em Experimental Animal – Faculty of Medicine – Federal University of Ceará number 113/07) that follows the rules of the Brazilian Committee on Animal Experimentation (COBEA).

Description of the meniscotomy and meniscectomy models

Mice were anesthetized with i.m. ketamine (50 mg/kg) and xylazine (10 mg/kg). After preparing for local aseptic surgery, the right knee joint was opened through a parapatellar medial incision with a scalpel. The patella was laterally displaced in order to expose the joint. Both the medial collateral and anterior cruciate ligaments were carefully transected in order to ease the access to the medial meniscus without damaging the articular cartilage. Following these procedures, groups of animals were then subjected to either transection of the medial meniscus, leaving a freed fragment inside the joint (meniscotomy group), or complete removal of the medial meniscus (meniscectomy group). In the meniscotomy group, the incision was made in the mid-body of the medial meniscus. In the meniscectomy group, the meniscus was freed from both the medial and lateral attachments trying to avoid damage to the underlying tibial cartilage.

The joint capsule and skin were sutured with Vycril (6-0) and mononylon (4-0) threads, respectively. A sham group was subjected to skin incision, patella displacement, and exposure of the joint without damage to ligaments or meniscus. A control group of animals was subjected to transection of both the medial collateral and anterior cruciate ligaments (ACLT/MCLT) without direct surgical damage to the meniscus. Naïve animals did not receive any manipulation. Those mice were used to compare differences concerning the pain behavior and histopathological parameters, as described below, comprising 10 animals for each of the five groups.

Assessment of pain behavior

Assessment of pain behavior (regarded as joint pain) was done daily using the electronic pressure-meter nociception paw test by an observer blinded to group allocation[16,17]. In this method, there is no direct stimulation to the affected joint. It is believed that the surgically-induced OA causes increase in sensitivity to a stimulus applied distal to that joint that would normally be innocuous. Animals were placed in acrylic cages (12 × 10 × 17 cm high) with a wire grid floor, 15 min before the beginning of the tests, in a quiet room. Stimulations were performed only when animals were quiet, without exploratory, urination or defecation movements and not resting on their paws. The electronic pressure-meter consists of a hand-held force transducer fitted with a polypropylene tip (Electronic von Frey aesthesiometer, Insight Equipamentos Científicos Ltda., Brasil). The polypropylene tip was applied perpendicularly to one of the five distal footpads of the right hind paw. The intensity of the stimulus was automatically recorded when the paw was withdrawn. The test was repeated three times, until less than a 1 g difference between measurements was obtained. Results were expressed as the mean value of three withdrawal threshold measurements (g).

Assessment of cell influx in joint aspirates

Animals subjected to the meniscotomy and meniscectomy procedures as well as sham-operated and naïve animals, as described above, were sacrificed 49 days after the surgical procedure, under terminal anesthesia. The synovial cavity of the knee joints was then washed with 0.05 ml saline containing 10 mM EDTA. The synovial fluids were collected by aspiration and total cell counts were performed using a Neubauer chamber. Differential cell counts were performed using the panoptic Instant Prov™ staining kit (New ProvBrasil™). After centrifuging (500 g/10 min), the supernatants were used for measuring the concentrations of NO using a commercially available kit (R & D Systems, São Paulo, Brazil).

Histopathology

The same groups of animals used for cell counts had their joint tissues excised for the histological study. After fixation in 10% v/v formaldehyde solution and decalcification (5% v/v formic acid in 10% v/v formaldehyde solution), the whole joint, comprising the distal femoral and proximal tibial extremities, was processed for paraffin-embedding and staining with hematoxylin-eosin (HE) and safranin-O. The material was serially sectioned at 5 μm in the sagittal plane of the articular surface, from the outer to the inner limits of the condyles. At every tenth section (50 μm apart), one section was removed for staining, with ten different sections for each sample. Analysis was done for both condyles and expressed as one result for each sample. Semi-quantitative histopathological grading was performed by two independent pathologists (VCCG, MMLP) blinded to group allocation according to the Osteoarthritis Research Society International (OARSI) histopathology grading and staging system[18]. The maximal possible final score (mean of measures made by the two pathologists) was 24. Results are expressed

as the median (interquartile range – IQR) values for each treatment group.

Three other groups of four animals each including a control group subjected to ACLT/MCLT, a meniscotomy and a sham-operated group were sacrificed 21 days after the surgical procedure, under terminal anesthesia. Total and differential cell counts were performed in the joint washes of these groups and joint tissues were excised, processed for histology, and evaluated using OARSI scoring system, as described above.

Drug treatments

After establishing that the meniscotomy procedure led to more severe damage (see below in Results section), pharmacological modulation was done solely in other groups of animals subjected to the meniscotomy model. In the pharmacological study, there were three groups of at least six animals. One of them was a sham group that received no pharmacological intervention throughout the study. A second group, at day 21, that had been subjected to meniscotomy, received a single indomethacin (2 mg/kg; s.c.) dose and another meniscotomy group, named non-treated (NT) group, received its vehicle (see below) s.c. At day 24, the group that received indomethacin was given the specific inducible nitric oxide synthase (iNOS) inhibitor 1400W (0.5 mg/kg s.c.) and the NT group received saline (s.c.). Pain behavior was evaluated 1 h post-dose. These compounds were administered sequentially, in that order, starting 21 days after the surgery. This strategy allowed the animals that received the treatments to redevelop the pain behavior, so that the paw withdrawal threshold returned to the levels prior to the administration of each compound.

In an attempt to study the analgesic potential of opioids, four groups of at least six animals subjected to meniscotomy received 1, 2 or 4 mg/kg morphine or saline and the pain behavior was measured after 1 h. Results represent pooled data from two experiments.

All compounds but indomethacin were dissolved in a 0.9% saline solution. Indomethacin was dissolved in a 5% $NaHCO_3$ 1 M solution and the pH was then adjusted to 7.0 with a 1 M HCl solution, just prior to administration. Naïve animals received saline i.p. Morphine was purchased from Cristalia Laboratórios, SP, Brazil. Indomethacin and 1400W were purchased from Sigma–Aldrich, SP, Brazil.

Statistical analysis

Results are presented as means ± 95% C.I. for pain behavior and cell counts or medians (IQR) for histology, of measurements made on at least six animals in each group. Assessment of normality of the pain behavior data was done using the D'Agostino-Pearson Omnibus test. Differences between drug treatment groups were compared using unpaired Student's *t*-test. Longitudinal analysis of pain behavior in each group was evaluated using repeated measures ANOVA; for multiple comparisons between means and medians we used one-way ANOVA followed by Tukey's test or Kruskal–Wallis test, respectively; $P < 0.05$ was considered as significant.

Results

Kinetics of pain behavior

Figure 1 shows ongoing records of daily pain behavior in mice subjected to either removal (meniscectomy) or transection (meniscotomy) of the medial meniscus, followed for 7 weeks. It can be clearly seen that the pain component, represented by a decreased mechanical paw withdrawal threshold, significantly developed in both groups during the first 14 days after surgery, compared to the sham group. However, following this period, there was a progressive increase in the mechanical threshold in mice subjected to meniscectomy. After 16 days, there is a statistically significant difference between both groups so that the meniscectomy group now displays significantly less pain behavior, as compared to the meniscotomy group, that persisted until the end of the experiment, at 49 days of observation. Animals subjected only to transection of the medial collateral and anterior cruciate ligaments, without damage to the meniscus, did also show a decrease of paw withdrawal threshold until 14 days after surgery that subsided at 21 days.

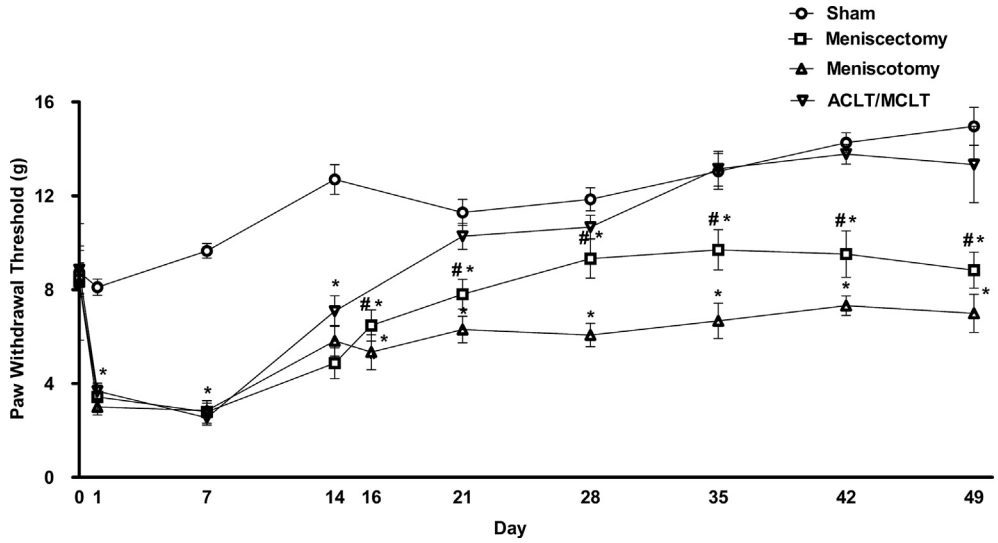

Fig. 1. Kinetics of the pain behavior in mice subjected to meniscectomy or meniscotomy. Mice were subjected to a sham operation, ACLT/MCLT or transection of the medial collateral and anterior cruciate ligaments followed by either meniscotomy meniscectomy. Pain behavior was evaluated using the von Frey procedure during 49 days. Results are expressed as the means ± 95% confidence intervals (CI) of groups of 10 animals; $^{*}P < 0.001$ compared to sham; $^{\#}P < 0.001$ compared to meniscotomy using one-way ANOVA followed by Tukey's test.

Analysis of cell influx into the joints

Figure 2 shows cell counts in the joint aspirates of mice subjected to ACLT/MCLT or ACLT/NCLT followed by either meniscectomy or meniscotomy, measured at 49 days after surgery. Analogous to what was observed with the pain behavior, there is a clear difference between the meniscectomy and meniscotomy groups, with a significant increase in cell numbers in the joint cavity only of the latter group, as compared to sham-operated animals ($P = 0.027$). These were almost exclusively mononuclear cells (>90%), mainly composed of lymphocytes and a few synovial cells.

Histopathological analysis of joint changes

Figure 3(A) and (B) shows the histological scores at both the femoral and tibial extremities of mice subjected to ACLT/MCLT, meniscectomy or meniscotomy, as compared to sham-operated animals. Both surgically-induced strategies significantly provoked lesions in the femoral (Fig. 3A) and tibial (Fig. 3B) extremities. It can also be seen that animals of the meniscotomy group displayed a tendency to have more severe lesions in both extremities. However, statistically significant higher scores were achieved only at the tibial plateau of the meniscotomy group, as compared to the meniscectomy group (Fig. 3B). Figure 4(A), (B), (C)and (D) are representative illustrations of the damage produced in sham-operated animals, mice subjected to ACLT/MCLT, meniscectomy or meniscotomy, respectively.

A group of four animals subjected to ACLT/MCLT followed by meniscotomy and sacrificed at 21 days had 9 (8–12) and 12 (6–14) median OARSI scores for the femur and tibia, respectively. Animals of the sham group had 0 (0–0) and 1 (0–2) median OARSI scores and those of the ACLT/MCLT group, also sacrificed after 21 days, had 6 (4–8) and 7 (4–8) median OARSI scores for the femur and tibia, respectively. A representative illustration of samples from animals sacrificed 21 days following a sham surgery, ACLT/MCLT or meniscotomy is shown in Figure 5(A–C), respectively. In addition to the cartilage damage seen in the meniscotomy group there was infiltration of mononuclear cells in the synovium, as shown in Fig. 5C, coupled to a mild (112 ± 82 cells/mm^3) influx of mononuclear cells in the joint lavage in this same group. Joint washes in the sham and ACLT/MCLT groups sacrificed after 21 days had 50 ± 82.5 and 50 ± 87 mononuclear cells/mm^3, respectively. Since OA changes in the meniscotomy group were already present at this time, we decided to perform drug studies starting 21 days after meniscotomy.

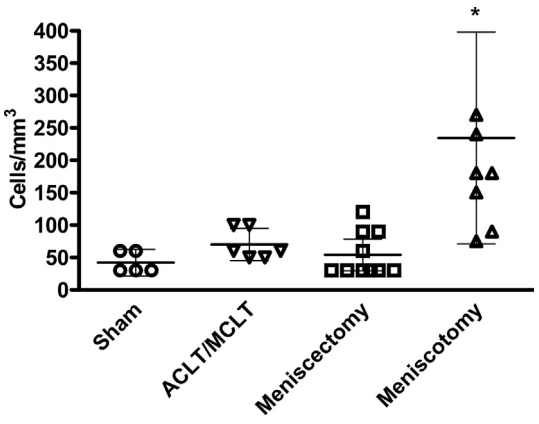

Fig. 2. Cell counts in joint exudates in mice subjected to meniscectomy or meniscotomy. Mice were subjected to a sham operation, ACLT/MCLT, meniscotomy or meniscectomy. Cell counts were assessed in joint lavage at 49 days after surgery. Results are expressed as the means ± 95% CI; $^*P = 0.027$ compared to sham using one-way ANOVA followed by Tukey's test.

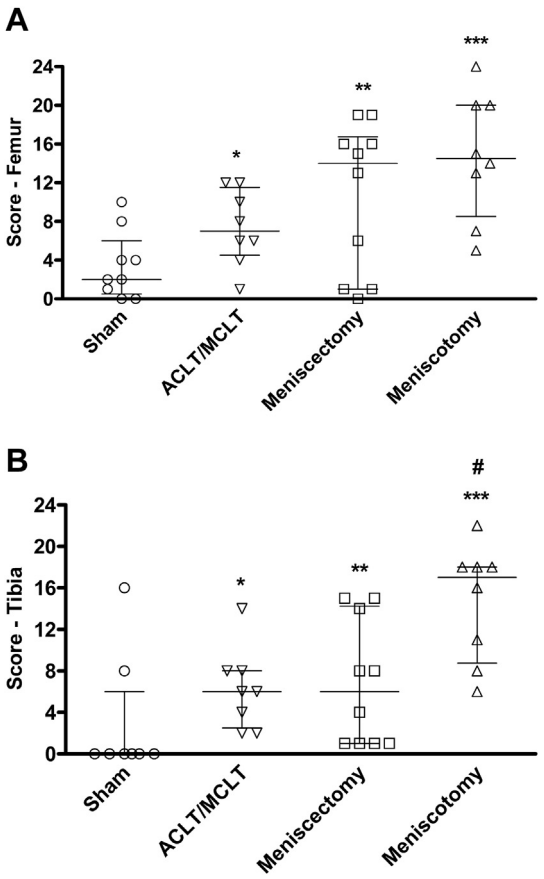

Fig. 3. Histopathological scores of the knees of mice subjected to meniscectomy or meniscotomy. Mice were subjected to a sham operation, ACLT/MCLT, meniscotomy or meniscectomy. Histological analysis was done using the OARSI scoring system at the distal femoral extremity (A) and tibial plateau (B), 49 days after surgery. Results are expressed as the median and IQRs; (A) $^*P = 0.0265$, $^{**}P = 0.0389$, $^{***}P = 0.0012$ compared to sham; (B) $^*P = 0.0292$, $^{**}P = 0.0165$, $^{***}P = 0.0021$ compared to sham; $^{\#}P = 0.0069$ compared to meniscectomy using Kruskal–Wallis test.

Effect of morphine on pain behavior

Since we obtained that the meniscotomy group displayed a greater reduction of paw withdrawal threshold, the pharmacological manipulation was then performed only in groups of mice subjected to meniscotomy. At day 24 following meniscotomy, pain behavior was significantly and dose-dependently reduced 1 h following intraperitoneal injection of morphine at various doses, as compared to a vehicle-treated group [Fig. 6(A)].

Effect of a cyclooxygenase inhibitor and a specific iNOS inhibitor on pain behavior

Therapeutic administration of indomethacin in mice subjected to meniscotomy (at day 21) significantly inhibited the decrease in paw withdrawal threshold [$P < 0.001$; Fig. 6(B)]. After restoration of the pain behavior, administration of the iNOS inhibitor 1400W (at day 24) did also significantly reduce the decrease in paw withdrawal threshold in meniscotomized mice [$P < 0.001$; Fig. 6(B)]. There was no significant difference between the analgesic result of the two compounds and the time-response is compatible with their half-lives.

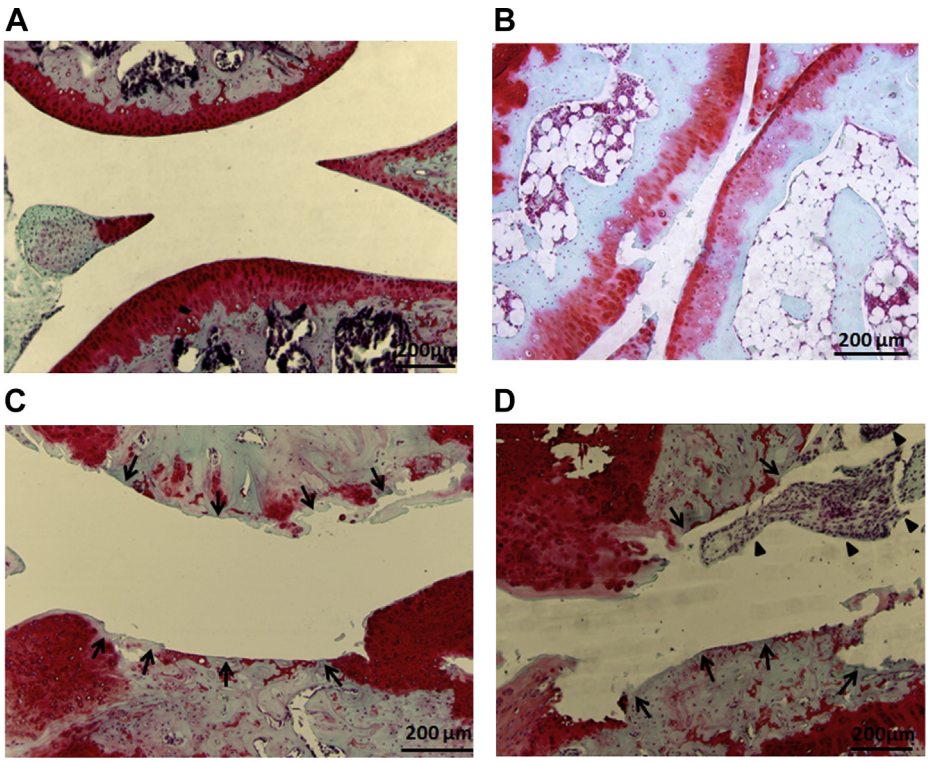

Fig. 4. Representative illustration of the synovial histopathology of the knees of mice subjected to meniscectomy or meniscotomy. Mice were subjected to a sham operation (A), ACLT/MCLT (B) meniscotomy (C) or meniscectomy (D). All animals were sacrificed at 49 days. While there is preservation of the cartilage and integrity of the meniscus in the sham-group, there is intense chronic synovitis in group D (arrow-head) as well as severe and extensive destruction of the cartilage, including exposure of the underlying subchondral bone in both groups C and D (arrows) (Safranin O – original magnification ×200).

Discussion

In the present study, focusing on pain behavior and structural damage, we compared groups of mice subjected to ACLT/MCLT followed by either meniscectomy or meniscotomy. There was no alteration in paw withdrawal threshold in animals of the sham group. A previous study using a similar sham group that had the joint washed prior to wound closure has shown development of a mild pain behavior[19]. We speculate that avoidance of any possible additional trauma apart from joint opening and patella subluxation and special care at wound closure might explain that no pain behavior was detected in our sham group.

The kinetics of the pain behavior of both meniscotomized and meniscectomized groups revealed a statistically significant increase in pain in both groups, as compared to a sham group. However, 16 days post-surgery, a significant difference in the pain behavior component appeared between those two groups, so that the group subjected to meniscectomy displayed a progressive reduction, as compared to the level registered in the animals of the meniscotomy group at the same time. Registration of threshold levels until 49 days post-surgery revealed that the meniscectomy group remained with significantly less pain, as compared to the meniscotomy group. Since both groups were also subjected to injury of the medial collateral and anterior cruciate ligaments, these data clearly show that leaving the damaged meniscus fragment inside the mice knees provoked a significant and sustained increase in the pain behavior, as compared to the strategy of completely removing the meniscus.

All groups displayed a decrease of the mechanical threshold, which captures pain arising distal from the injured joint thus reflecting central sensitization[17], until 14 days after surgery. After this time, the mechanical threshold of animals from both groups subjected to meniscal lesion was significantly different from those subjected only to transection of the ligaments. By 21 days, the pain behavior in those animals subjected solely to transection of the ligaments was no longer detected. The possibility that surgical trauma, including joint manipulation and hemorrhage provoking a local inflammatory reaction accounted for this early pain component is very likely. A recent study comparing gait changes in sheep subjected to three different meniscal lesions at 2.5, 8, and 12 weeks post-surgery did not find differences of both pain and histological parameters regardless of the lesion provoked[20]. Our strategy compared the pain behavior in ongoing OA to baseline threshold levels of the same group, thus minimizing bias[21]. Additionally, the mechanical threshold is not influenced by gait changes. Therefore, we conclude that damage to the meniscus is the main reason for sustained pain behavior development in this surgically-induced OA model. Furthermore, leaving the torn meniscus inside the joint provoked a sustained pain behavior that is significantly increased as compared to a strategy of completely removing the meniscus. Curiously, patients with a torn meniscus subjected to its surgical removal usually recover from the pain after the procedure, even though the damage to the joint may progress leading to OA changes in the future[4].

The reasons for the difference observed in both groups are not straightforward. However, we speculate that leaving the meniscus fragment inside the joint provokes more instability thus resulting in sustained pain. Among the possible mechanisms, we could propose the stimulation of nociceptor fibers present in the rim of the damaged meniscus and the pressure applied to the underlying subchondral bone[22].

Pharmacological modulation by classical analgesic and/or anti-inflammatory compounds aids in validating experimental pain models[23]. The mechanical threshold was significantly increased by the administration of indomethacin, a classic non-selective

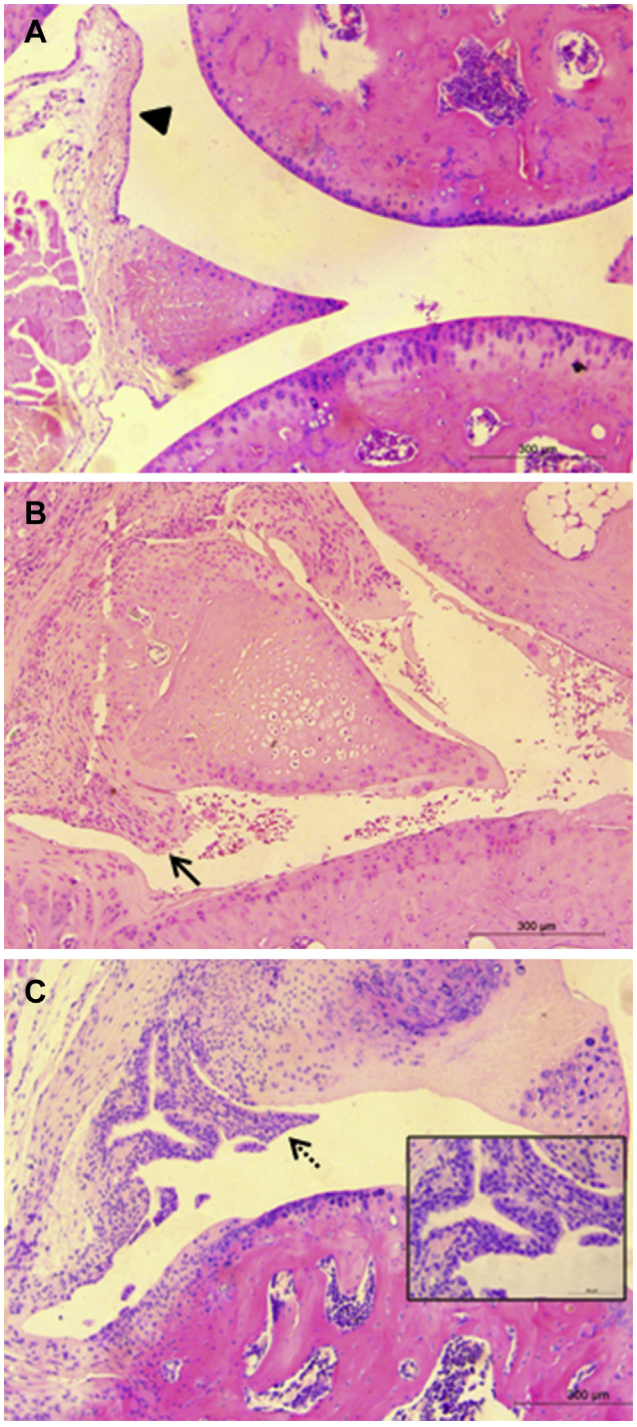

Fig. 5. Representative illustration of the histopathology of the knees of mice 21 days after being subjected to meniscotomy. Mice were subjected to a sham operation (A), ACLT/MCLT (B) or ACLT/MCLT followed by meniscotomy (C). All animals were sacrificed at 21 days following the surgical procedure. Well-preserved smooth cartilage surface, organized chondrocytes, and intact meniscus in (A), with no alterations in the synovium (arrow-head). Cartilage fissures occur in B, with disoriented chondrocytes and scattered dead chondrocytes. Cartilage surface is damaged in less than 25% of the extension and a mild cellular reaction is seen in the synovium in (B) (solid arrow). A grade 3 OARSI score is shown in the cartilage in (C), with vertical fissures (clefts). Chondrocyte proliferation, as well as dead chondrocytes, is prominent coupled to cartilage erosion with delamination areas and excavations involving more than 25% of the horizontal extent thus representing a stage 3 OARSI score. There is also thickening of the synovial membrane, with infiltration of mononuclear cells (inset) and a reparative tissue seems to invade the joint space in (C) (dashed arrow) (HE – original magnification ×100; inset – original ×200).

cyclooxygenase inhibitor. Given the well-known participation of prostanoids in pain modulation in both experimental and human OA[24,25], this result could of course be anticipated. However, it adds to validating the pain assessment method employed besides illustrating reproducibility. A similar increase in the mechanical threshold was observed when we administered a selective iNOS inhibitor. It should be remarked that these were therapeutic rather than prophylactic interventions made in the same group of animals. Thus, it may imply clinical relevance. Our purpose at this moment was to demonstrate that the pain behavior subsides following a "pain-killer" administration returning after the compound is no longer pharmacologically active. In addition to further substantiating the multimediated character of pain mechanisms operating in this OA model, this strategy clearly reduces the number of animals used in the experiments thus complying with the strictest ethical rules on animal experimentation.

Prostanoids are classical mediators of inflammatory pain. Actually, the current OARSI recommendations for pain relief in OA subjects include the use of cyclooxygenase inhibitors[25]. Therefore, the pain reduction promoted by indomethacin in this meniscotomy model adds support to its reproducibility to study pain mechanisms in experimental OA. A similar interpretation could be applied to the pain inhibition provided by the 1400W compound. Reactive nitrogen/oxygen-derived species have long been recognized as participating in joint damage in OA[26]. However, a double-blind placebo controlled trial using a selective iNOS inhibitor in humans with knee OA failed to demonstrate improvement of either symptoms or structural damage[27]. Although our data add support to a role for NO in pain modulation, the clinical relevance remains to be demonstrated.

In another attempt to further characterize the joint pain, we showed that morphine virtually abolished the pain behavior in meniscotomized animals. The analgesic efficacy of opioid agonists in joint pain in humans with OA has long been recognized[28]. Actually, opioid agonists are recommended as alternative options to non-steroidal anti-inflammatory drugs as "pain-killers" to treat OA patients[25]. However, concerns about safety regarding the use of those compounds have been raised[29].

The cell influx into the joints in human OA is mild and it is usually composed of lymphocytes and other mononuclear cells[30,31]. Similar to what we observed with the pain component, a significant difference between the meniscectomy and meniscotomy groups was observed regarding the number of cells in the joint lavage present at 49 days after surgery. Assessing cell counts in the joint lavage probably reflects local synovial changes and this alteration further strengthens our proposal that the group subjected to meniscotomy had a more severe lesion.

The histopathological changes in the femoral extremities did not differ between the meniscotomy and meniscectomy groups. However, there was a mild, though significant, difference in the changes found at the tibial extremities, being more severe in the former group. Some animals of the sham group displayed relatively high histopathological scores. Those mice were subjected to joint opening. Despite all the care during surgery, we cannot exclude the possibility of inadvertent damage to joint structures to explain this unexpected result. On the other hand, low scores did also occur in mice subjected to meniscal damage. Despite this variability, we were able to demonstrate significant differences between the meniscectomy and meniscotomy groups thus arguing for the relevance of the meniscal lesion provoked.

Contrary to our data, the recent study in sheep subjected to three different meniscal lesions mentioned above did not find significant differences among the groups[20]. These results reveal dissociation between pain severity and histological lesion, which is similar to what happens in humans with knee OA. Actually,

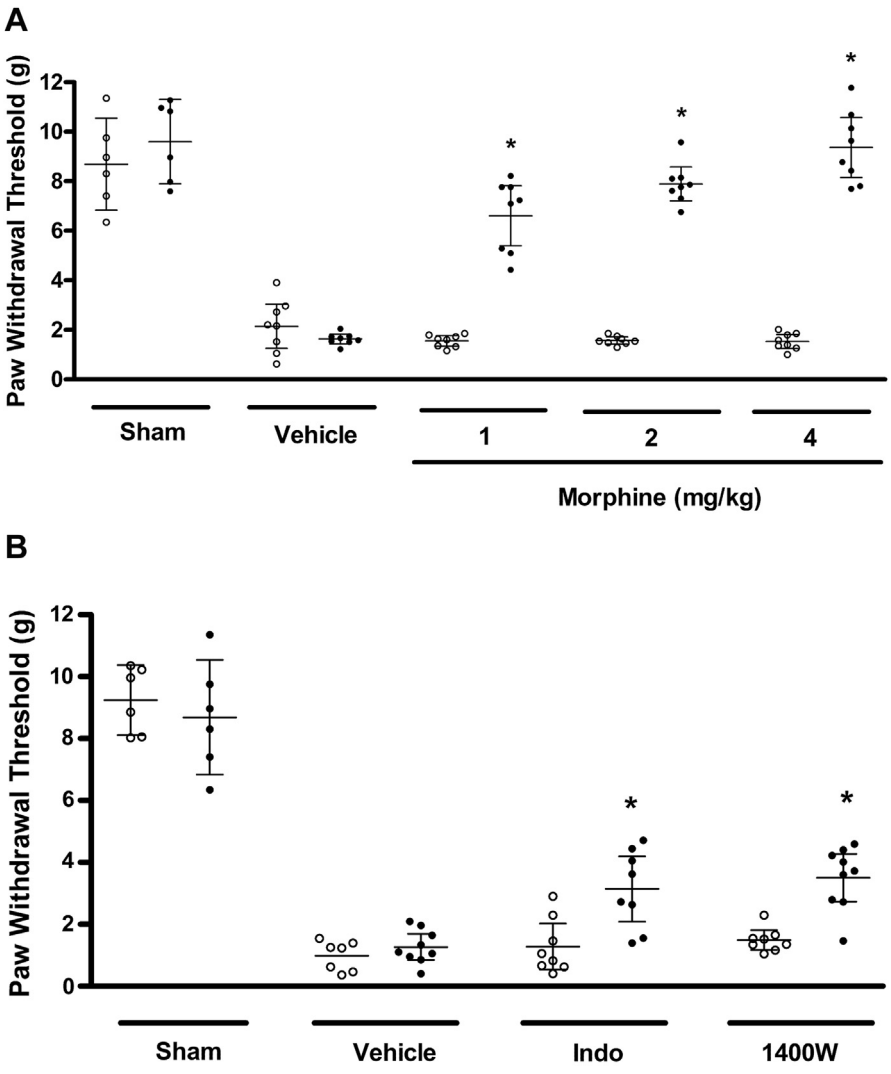

Fig. 6. Effect of analgesics on the pain behavior in mice subjected to meniscotomy. Mice were subjected to a sham operation or ACLT/MCLT followed by meniscotomy. Pain behavior was evaluated using the von Frey procedure. (A) Groups received morphine (1, 2 or 4 mg/kg; i.p.) or saline; (B) Groups received indomethacin (2 mg/kg; s.c.) or vehicle at day 21 after surgery followed by 1400W (0.5 mg/kg; s.c.) or vehicle at day 24 after surgery. Data represent the means ± 95% CI of paw withdrawal threshold. Open and closed symbols represent values pre and post dose, respectively; $^{*}P < 0.001$ compared to pre-dose using Student's *t*-test.

incidental meniscal lesions were reported to occur in human subjects from 50 to 90 years of age, regardless of the presence of joint symptoms[32].

The present study has some limitations. There were high scores in the histopathology analysis of two animals in the sham group, which are hard to explain. We believe that inadvertent surgical trauma leading to bleeding into the joint could provoke joint OA lesions in those mice in a way similar to what happens in patients affected by hemophilia[33]. Also, the great variability in the histological scores among groups may at least in part have been caused by difficulties in performing the surgery. Previous studies have reported discrepancy in data obtained using the ACLT and DMM models in mice in studies from different groups. Surgical technique has also been proposed to influence those results[14]. Additionally, we have to consider that the use of different strains, lack of standardization of tissue sectioning, and scoring system can contribute to explain the apparently discrepant results obtained from various groups using similar models[9,14,34].

Comparison across different studies should take into consideration the standardization of procedures[21]. Our data reinforce the importance of detailed description of the joint structures damaged in animal models of OA regardless of the size of the animals used. Small samples size represents a difficulty to evaluate histological changes in mice models of OA. On the other hand, the possibility of pharmacological manipulation and use of genetically modified animals represent a major advantage of mice models. Our results reinforce that detailed description of the lesions provoked in animal models is crucial in order to appropriately compare results obtained by different groups.

Author contributions

ACRL, FQC, TMC, FACR contributed in the conception of the protocol; VCCG, MMLP performed the histological analysis; ACRL, MAAT, RMN, ACMDP performed the pain behaviour studies; MAAT, ACRL, ACMDP, FACR performed data analysis; ACRL, VCCG, ACMDP, TMC, FQC, FACR wrote and revised the manuscript.

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Competing interest statement
The author and co-authors declare that there are no conflicts of interest to disclose concerning the publication of this article.

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